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THE VOLATILE ACIDS PRODUCED BY STARTERS AND BY ORGANISMS ISOLATED FROM THEM

By B. W. Hammer and F. F. Sherwood

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THE VOLATILE ACIDS PRODUCED BY STARTERS AND BY THE ORGANISMS ISOLATED FROM THEM

By B. W. Hammer and F. F. Sherwood

The work done in various laboratories during the last few years has shown that starters, instead of being pure cultures of *Streptococcus lactis,* as was at one time commonly supposed, contain two or more organisms, at least one of which produces considerable amounts of volatile acid. Good starters have regularly been found to yield a high and rather definite amount of volatile acidity but natural souring and sometimes poor starters also give a high volatile acidity, so that such an acidity is no guarantee as to the quality of a starter. This indicates that while the amount of volatile acid produced is important with a starter, the character of the volatile acids must also be considered and suggests the desirability of knowing what kinds of acids are produced in starters and in pure cultures of the starter organisms. The work herein reported represents an attempt to secure information along this line and also on the kinds of acids produced during prolonged natural souring such as occurs in old cream.

METHODS USED

The solutions of volatile acids studied were secured by distilling the fermented milk or cream with steam after the addition of a small amount of N/1 H_2SO_4 to free any volatile acids that might have been fixed by the milk constituents. With the starters a liter of the fermented material was commonly distilled in a five liter flask after the addition of 45 c.c. of N/1 H_2SO_4, while with the pure cultures of organisms it was more common to distil 250 cc. of the milk cultures in a two liter flask after adding 15 cc. N/1 H_2SO_4; in both cases the usual amount of distillate collected was 1 liter. In order to secure larger amounts of volatile acid with the starters two distillations were commonly made at the same time and the distillates mixed while with the pure cultures distillates were also frequently combined.

Two methods were used in determining the kinds of volatile acids present in the distillates, the estimation of the percent Ba* in the barium salt and a modified Duclaux method.

*Before deciding on the use of the barium salt, the silver salt method (Barthel Chr. 1910—Methods used in the Examination of Milk and Dairy Products, p. 219) was tried but under the conditions used was not as satisfactory as the barium salt method.

TABLE I—PERCENT Ba IN SALTS PREPARED FROM COMMERCIAL ACETIC AND PROPIONIC ACIDS

	% Ba in barium salt			
	A	B	Average	Theoretical
Ba acetate:				
Trial 1	53.44	53.37	53.405	53.78
Trial 2	53.73	53.59	53.66	
Trial 3	53.46	53.40	53.43	
Trial 4	53.78	53.78	53.78	
Ba propionate:				
Trial 1	48.60	48.29	48.445	48.46
Trial 2	48.47	48.50	48.485	
Trial 3	48.53	48.48	48.505	
Trial 4	48.44	48.41	48.425	
Ba butyrate				44.10

The usual procedure in determining the percent Ba was to titrate 100 cc. of the distillate with N/10 Ba $(OH)_2$, using phenolphthalein as an indicator, and then to add a little less than the calculated amount of $Ba(OH)_2$ to the remaining 900 cc. of distillate, the 100 cc. portion to which indicator had been added being discarded. The aqueous solution of the barium salt was concentrated on the water bath to about 50 cc. and filtered. After evaporating to dryness on the water bath the salt was recrystallized, dried at 100° C. and the percent Ba determined as follows: Two portions of about ½ gram each were weighed out, dissolved in from 75 to 100 cc. of hot water, heated to boiling and a slight excess of normal H_2SO_4 slowly added while boiling the solution. After digesting over night on the hot plate (under a watch glass so that the free volatile acids would not be lost since a Duclaux determination was to be run on them), the $BaSO_4$ was filtered off, ignited and weighed. From the weight of $BaSO_4$ and the weight of the original salt the percent Ba in the latter was calculated. Table I shows the results obtained by this method with supposedly pure acetic and propionic acids, from commercial sources.

The filtrates from the Ba determinations were used for the Duclaux method which was carried out according to the modification of Boekhout and Ott de Vries.[1] The volume of the solution being distilled was kept constant at 110 cc. and distilled at the rate of 100 cc. in about 45 minutes. The distillate was col-

1. (Dept. Landb.), Nijv. en Handel (Netherlands), Verslag en Meded. Dir. Landb. 5, 12 (1916) Thru Exp. Sta. Record 37, p. 207.

lected in ten cc. fractions and each fraction titrated with N/10 Ba $(OH)_2$ solution using phenolphthalein as an indicator. This method is based upon the fact that each volatile fatty acid has a constant rate of vaporization when distilled under given conditions. For example, if a definite amount of acid is subjected to distillation, the amount that passes over in each successive fraction bears a definite relationship to the total acidity of the distillate. Constants were determined for acetic and propionic acids using about a 0.5 percent solution since this was approximately equivalent to the strength of the unknown solutions worked with; these are given in table II, where A represents the titration figure for each successive 10 cc. of distillate, B the sum of these figures for a given amount of distillate, and C the value given in B calculated as the percentage of the titration figure for the total 100 cc. of distillate. The values for propionic acid are higher than the values for acetic acid indicating that propionic acid distills more rapidly than acetic acid.

The volatile acids worked with often gave constants which fell in between those for acetic and propionic acids. When the constants were much nearer those of acetic than those of propionic acid the mixture was considered as mainly acetic plus a small amount of propionic acid and when the values obtained agreed less closely with those for acetic acid the mixture was considered to be acetic plus large amounts of propionic acid. Table III illustrates the type of constants that were considered to indicate a mixture of acetic acid plus a small amount of pro-

TABLE II—DUCLAUX VALUES FOR ACETIC AND PROPIONIC ACIDS

Acetic Acid
cc. of distillate

	10	20	30	40	50	60	70	80	90	100
A*	2.45	2.30	2.20	2.10	2.05	1.90	1.85	1.80	1.75	1.70
B**	2.45	4.75	6.95	9.05	11.10	13.00	14.85	16.65	18.40	20.10
C***	12.19	26.63	34.57	45.02	55.22	64.68	73.89	82.83	91.54	100

Propionic Acid
cc. of distillate

	10	20	30	40	50	60	70	80	90	100
A*	3.75	3.35	3.05	2.85	2.75	2.60	2.35	2.15	2.00	1.80
B**	3.75	7.10	10.15	13.00	15.75	18.35	20.70	22.85	24.85	26.65
C***	14.07	26.65	38.08	48.77	59.10	68.87	77.67	85.74	93.26	100

*A=cc. N/10 Ba $(OH)_2$ required for successive 10 cc. fractions.
**B=sum of values in A for a given amount of distillate.
***C=values given in B calc. as percent of the titration values for the 100 cc. of distillate.

TABLE III—CONSTANTS ILLUSTRATING ACETIC ACID PLUS A SMALL AMOUNT OF PROPIONIC

Source of material	cc. of distillate									
	10	20	30	40	50	60	70	80	90	100
Starter	12.39	24.04	35.22	45.39	55.46	65.34	74.67	83.73	91.94	100
Starter	12.45	24.28	35.27	46.06	55.14	66.19	75.95	83.70	91.94	100
Starter	12.65	24.53	35.65	45.99	55.95	65.54	74.73	83.54	91.98	100

pionic acid while table IV illustrates the constants considered to indicate a mixture of acetic acid with large amounts of propionic acid. While the Duclaux method as employed cannot be considered entirely satisfactory as a means of determining the kinds of volatile acid present in a mixture, its use seemed desirable in order to confirm the results secured with the method of determining the percent Ba in the barium salt.

RESULTS SECURED

The types of volatile acids produced in starters ripened for long periods are illustrated by table V. These results were obtained during the preliminary work when the methods to be employed were being studied; the long ripening periods were used because considerable quantities of volatile acids were desired and they were most easily secured from highly ripened starters. The data presented show that with starters ripened for considerable periods the barium values on the volatile acids were over 53 percent in all cases, but not up to the values secured with pure acetic acid; this suggests that the acid is very largely acetic with a small amount of propionic. The Duclaux values indicate the same thing. It is of interest to note how little variation is evident in the barium values obtained on a number of starters from a wide variety of original sources, when these were ripened for considerable periods.

Table VI gives the results obtained on a number of starters when two determinations of the types of volatile acids present

TABLE IV—CONSTANTS ILLUSTRATING ACETIC ACID PLUS A LARGE AMOUNT OF PROPIONIC

Source of material	cc. of distillate									
	10	20	30	40	50	60	70	80	90	100
Culture S. citrovorus	13.38	25.97	37.21	47.67	57.18	66.07	77.82	83.33	91.85	100
Starter	13.43	25.37	37.31	48.51	58.95	68.66	77.61	85.82	93.18	100
Starter	12.96	25.92	37.03	48.22	57.41	68.67	77.93	85.26	92.60	100

TABLE V—BARIUM AND DUCLAUX RESULTS OBTAINED ON VOLATILE ACIDS FROM HIGHLY RIPENED STARTERS

Starter	Incubation	Acidity of 1 *l.* distillate from 1 *l.* starter (steam distillation)	% Ba in barium salt			Results of Duclaux
			Det. A.	Det. B.	Average	
D 83		60.5 cc. N/10	53.02	53.15	53.085	Acetic plus trace of propionic
D 83	3 d. 21° C.	54.0 " "	53.08	53.11	53.095	Acetic plus trace of propionic
Hansen	2 d. 21° C.	53.0 " "	53.10	53.10	53.10	Acetic plus trace of propionic
Champion	2 d. 21° C.	63.0 " "	53.27	53.27	53.27	Acetic plus trace of propionic
Ericsson	1 d. 21° C.	60.0 " "	53.23	53.21	53.22	Acetic plus trace of propionic
D 52	2 d. 21° C.	58.0 " "	53.30	------	53.30	Acetic plus trace of propionic
Hansen	2 d. 21° C.	53.0 " "	53.02	53.09	53.055	Acetic plus trace of propionic

were made at different times, the object being to see whether the volatile acids were the same when the acidity of the starter was low as when it was high. The data presented show that with high acidities in the starter, the barium values on the volatile acids were essentially the same as those given in table V, being either above 53 percent or very close to this value in all cases. In general with the lower acidities in the starters, lower barium values were secured; in two instances with low acidity starters the barium values were only slightly over 51 percent and in two others only slightly over 52 percent. Since a low total acidity in the starter was accompanied by a low volatile acid production, low barium values were obtained with low volatile acidities. It is evident then that when a starter has produced only a low total and volatile acidity, the volatile acids have lower barium values than when the acidities are high and accordingly include more volatile acid of a type higher than acetic. The data presented indicate also that in general there was less change in the type of volatile acid in the two determinations when the first was on starter having a total acidity near 0.8 percent than when it was on a starter having a lower total acidity; that is, the influence of 0.1 percent in the total acidity of the starter on the type of volatile acid was less near the maximum acid production than it was at a lower acidity. The Duclaux results reported in table VI confirm the conclusions drawn from the barium values.

The results given in table VII which were obtained at a later date and to a large extent on a different lot of starters than those given in table VI are in the main confirmatory of those presented in the latter table. The same high barium values were secured on starters ripened to high acidities and the same general relationship between low total (or volatile) acidities and low barium values is evident. In the two instances where three determinations were made on a lot of starter instead of only two

TABLE VI—BARIUM AND DUCLAUX VALUES OBTAINED ON STARTERS AT DIFFERENT STAGES IN THE RIPENING

Starter		Time incubated at 21° C.	Acidity	Acidity of 1 *l.* dist. from 900 cc. starter (steam distillation)	% Ba in barium salt			Results of Duclaux
					Det. A	Det. B	Average	
Champion,	1st det.	21 hr.	.79	40.0 cc. N/10	53.10	53.15	53.125	Acetic plus trace of propionic
	2d det.	29 "	.97	47.5 " "	53.25	53.10	53.175	Acetic plus trace of propionic
Ericsson,	1st det.	15 "	.67	22.0 " "	51.09	50.95	51.02	Acetic plus small amount of propionic
	2d det.	24 "	.93	53.0 " "	53.13	53.16	53.145	Acetic plus trace of propionic
Hansen,	1st det.	15 "	.74	21.5 " "	52.62	52.86	52.74	Acetic plus small amount of propionic
	2d det.	24 "	.97	45.5 " "	52.94	52.93	52.935	Acetic plus small amount of propionic
D103,	1st det.	12 "	.58	15.5 " "	51.17	51.26	51.215	Acetic plus large amount of propionic
	2d det.	21 "	.77	47.5 " "	53.21	53.20	53.205	Acetic plus trace of propionic
Ericsson,	1st det.	14 "	.75	26.0 " "	53.02	52.94	52.98	Acetic plus trace of propionic
	2d det.	24 "	.96	50.0 " "	53.51	53.60	53.555	Acetic plus slight trace of propionic
Champion,	1st det.	17 "	.77	26.0 " "	52.84	52.69	52.765	Acetic plus small amount of propionic
	2d det.	24 "	.80	46.0 " "	53.40	53.46	53.43	Acetic plus trace of propionic
D103,	1st det.	13 "	.58	30.5 " "	52.30	52.26	52.28	Acetic plus small amount of propionic
	2d det.	20 "	.82	46.0 " "	52.83	52.83	52.83	Acetic plus small amount of propionic
D104,	1st det.	15 "	.76	43.0 " "	53.27	53.22	53.245	Acetic plus trace of propionic
	2d det.	24 "	.84	47.0 " "	53.20	53.18	53.19	Acetic plus trace of propionic
Champion,	1st det.	15 "	.68	20.5 " "	52.14	52.16	52.15	Acetic plus small amount of propionic
	2d det.	----------	.86	45.5 " "	52.95	53.02	52.985	Acetic plus small amount of propionic

TABLE VII—BARIUM AND DUCLAUX VALUES OBTAINED ON STARTERS AT DIFFERENT STAGES OF RIPENING

Starter	Time incubated at 21° C.	Acidity	Acidity of 1 *l.* dist. from 1 *l.* starter (steam distillation)	% Ba in barium salt			Results of Duclaux
				Det. A	Det. B	Average	
D104, 1st det.	15 hr.	.84	35.0 cc.	52.55	52.66	52.605	Acetic plus small amount of propionic
2d det.	20 "	.88	52.5 "	52.84	52.98	52.91	Acetic plus small amount of propionic
D99, 1st det.	17 "	.88	29.0 "	52.34	52.36	52.35	Acetic plus small amount of propionic
2d det.	22 "	.92	48.0 "	53.02	53.02	53.02	Acetic plus trace of propionic
D103, 1st det.	14 "	.67	17.0 "	50.18	50.48	50.33	Acetic plus large amount of propionic
2d det.	19 "	.86	40.5 "	52.95	53.14	53.045	Acetic plus small amount of propionic
D92, 1st det.	14 "	.76	27.0 "	51.52	51.63	51.575	Acetic plus small amount of propionic
2d det.	19 "	.89	50.0 "	53.07	53.15	53.11	Acetic plus trace of propionic
D115, 1st det.	14 "	.73	21.5 "	51.68	51.80	51.74	Acetic plus small amount of propionic
2d det.	19 "	.83	40.5 "	52.97	53.07	53.02	Acetic plus trace of propionic
D83, 1st det.	16 "	.84	25.0 "	52.79	52.84	52.815	Acetic plus small amount of propionic
2d det.	21 "	.87	51.5 "	53.23	53.33	53.28	Acetic plus trace of propionic
D103, 1st det.	14 "	.80	30.0 "	52.91	53.09	53.00	Acetic plus trace of propionic
2d det.	19 "	.85	54.0 "	53.13	53.25	53.19	Acetic plus trace of propionic
D104, 1st det.	15 "	.75	26.0 "	51.60	51.64	51.62	Acetic plus small amount of propionic
2d det.	20 "	.80	49.0 "	53.47	53.62	53.545	Acetic plus slight trace of propionic
3d det.	39 "	.95	62.5 "	53.20	53.27	53.235	Acetic plus slight trace of propionic
D23, 1st det.	14 "	.68	15.0 "	50.42	50.48	50.45	Acetic plus large amount of propionic
2d det.	20 "	.81	34.0 "	51.82	51.46	51.64	Acetic plus small amount of propionic
3d det.	38 "	1.05	51.2 "	53.37	52.52	53.445	Acetic plus trace of propionic
D104, 1st det.	15 "	.72	44.5 "	51.18	51.13	51.155	Acetic plus small amount of propionic
2d det.	20 "	.85	53.5 "	53.13	53.15	53.14	Acetic plus trace of propionic
D122, 1st det.	14 "	.70	21.3 "	50.47	50.60	50.535	Acetic plus large amount of propionic
2d det.	38 "	.94	43.5 "	52.71	52.60	52.655	Acetic plus small amount of propionic

TABLE VIII—BARIUM AND DUCLAUX VALUES OBTAINED ON FIRST AND SECOND LITER OF DISTILLATE FROM STARTER

First liter distillate

Starter	% Ba in barium salt			Results of Duclaux
	Det. A.	Det. B.	Average	
D104, 1st det.	51.60	51.64	51.62	Acetic plus small amount propionic
2d det.	53.47	53.62	53.545	Acetic plus slight trace propionic
3d det.	53.20	53.27	53.235	Acetic plus slight trace propionic

Second liter distillate

Starter	% Ba in barium salt			Results of Duclaux
	Det. A.	Det. B.	Average	
D104, 1st det.	51.62	51.56	51.59	Acetic plus small amount propionic
2d det.	53.26	53.31	53.285	Acetic plus trace propionic
3d det.	53.21	53.32	53.265	Acetic plus trace propionic

the results simply tend to confirm those secured where two determinations were used; in one instance the third determination gave a slightly lower barium value than the second but the difference is too small to be of any significance while in the other instance there was a rise in the barium value from the first to the third determination. The Duclaux results reported in table VII again confirm the conclusions drawn from the barium values.

The data presented in tables VI and VII indicate that at a given acidity starters may show quite different barium values. The differences along this line seem to be as great in different lots of starter inoculated from the same culture as in lots of starter inoculated with different cultures. It seems reasonable to conclude that the change from the volatile acids giving a low barium value (acetic with considerable propionic) to those giving a somewhat higher barium value (acetic with only a small amount of propionic) does not always occur at exactly the same total acidity of the starter.

With the series of three determinations that were made on one lot of starter 104 and reported in table VII barium and Duclaux values were also secured on a second liter of distillate from each determination. These are given in table VIII together with the barium and Duclaux values for the first liter of distillate from each determination for comparison. The data

show a close agreement between the values for the first and second liters in all three comparisons and suggest that the first liter of distillate is representative of the volatile acids produced in a starter and can be used for studies on the kinds of volatile acids formed.

The variation in the volatile acids produced at different periods during the ripening of a starter indicates that the different groups of organisms may not produce the same volatile acids, and suggests a consideration of the volatile acids formed by *S. lactis* and by the associated organisms in pure cultures.

Material for the determination of the kinds of volatile acids produced by *S. lactis* was secured by combining distillates obtained from cultures of *S. lactis* that were being checked to be certain of their low volatile acid production. While a distillate from a culture of one organism might have been preferable to the material used, the work involved in securing such a distillate with the low volatile acid producing *S. lactis* cultures seemed to justify the other procedure. The results secured on the *S. lactis* cultures are given in table IX; from these it will be seen that the pure cultures of *S. lactis* gave low barium values, comparable to those secured on a starter rather early in the ripening period. These barium values indicate that the volatile acid produced by *S. lactis* is not largely acetic[2] as has been suggested in some instances but is acetic with some higher acid presumably propionic included with it in considerable amounts. The Duclaux values confirm the conclusions drawn from the barium values.

TABLE IX—BARIUM AND DUCLAUX VALUES OBTAINED ON DISTILLATES FROM CULTURES OF S. LACTIS

Number of distillates mixed to supply acids	Average volatile acidities* of cultures S. lactis.	% Ba in barium salt			Results of Duclaux
		Det. A	Det. B	Av.	
2	8.2	51.34	51.77	51.555	Acetic plus small amount of propionic
2	10.7	50.48	50.59	50.535	Acetic plus large amount of propionic
4	10.7	51.82	51.74	51.78	Acetic plus small amount of propionic
2	11.0	50.23	50.49	50.36	Acetic plus large amount of propionic
2	7.5	50.64	50.93	50.785	Acetic plus large amount of propionic
4	7.5	50.34	50.49	50.415	Acetic plus large amount of propionic
4	8.1	51.86	51.90	51.88	Acetic plus small amount of propionic
6	7.4	50.78	50.71	50.745	Acetic plus large amount of propionic
4	6.0	50.54	50.31	50.425	Acetic plus large amount of propionic
4	7.6	49.63	49.86	49.745	Acetic plus large amount of propionic
4	7.7	51.46	51.28	51.37	Acetic plus small amount of propionic
9	8.7	50.12	50.24	50.18	Acetic plus large amount of propionic
6	7-1	49.98	50.31	50.145	Acetic plus large amount of propionic

*cc. of N/10 alkali required to neutralize the first liter distillate obtained by distilling 250 grams with steam after adding 15 cc. of approx. N/1 H_2SO_4.

2. See Evans, Alice C. A Study of the Streptococci Concerned in Cheese Ripening. Jr. Agr. Res. 13, Ap. 22, 1918, p. 235, for review of literature and original data.

In determining the volatile acids produced by the organisms associated with *S. lactis* in starters, various kinds of material were employed. In two instances distillates secured from milk cultures of *S. paracitrovorus* were used, while in the remainder distillates from cultures of *S. citrovorus* or *S. paracitrovorus* grown in milk to which citric acid had been added were employed. The addition of the citric acid increased very materially the amount of volatile acid produced and made it easier to secure sufficient volatile acid for the preparation of the barium salts in satisfactory amounts. The results secured with the associated organisms are presented in table X. The barium values with these organisms were higher than the values secured with *S. lactis* and approximated the values obtained with starters that had been allowed to develop considerable amounts of acid. It is accordingly evident that the associated organisms produce a volatile acidity that is largely acetic and thus quite different than the volatile acidity produced by *S. lactis.* The type of volatile acids produced by the associated organisms accounts for the type of volatile acids produced in starters that have developed considerable total acidity, which cannot be accounted for by the action of *S. lactis,* and thus affords further proof of the importance of the associated organisms in a starter.

In order to compare the volatile acids present in old cream soured naturally with those present in starter, the volatile acids were distilled from old cream with steam and the barium and Duclaux values determined. The old off-flavored cream was secured by holding cream at room temperature for varying lengths of time. The results obtained, which are given in table XI, show that the barium values were very different than the values secured on starters, as would be expected from the odor of the lots of old cream. The barium values are low indicating that acids higher than acetic must be present in considerable amounts. Some of the barium values are so low that if a certain amount of acetic acid was present as was probably the case, they could not be accounted for by the remainder of the volatile acid being propionic so that some higher acid probably butyric would necessarily be present. The Duclaux values on the distillates from old cream confirm the conclusions drawn from the barium values.

TABLE X—BARIUM AND DUCLAUX VALUES OBTAINED ON DISTILLATES FROM CULTURES OF ORGANISMS ASSOCIATED WITH S. LACTIS IN STARTERS

Source of Material	Volatile acidities* of cultures	% Ba in barium salt			Results of Duclaux
		Det. A	Det. B	Av.	
3 distillates from milk inoc. with S. paracitrovorus	23.0 av.	52.64	52.73	52.685	Acetic plus small amount of propionic
5 distillates from milk inoc. with S. paracitrovorus	26.1 av.	52.50	52.58	52.54	Acetic plus small amount of propinic
S. paracitrovorus in milk plus citric acid	81.5	53.63	53.53	53.58	Acetic plus trace of propionic
S. paracitrovorus in milk plus citric acid	75.7	53.35	53.30	53.325	Acetic plus small amount of propionic
S. paracitrovorus in milk plus citric acid	58.0	53.40	53.52	53.46	Acetic plus small amount of propionic
S. paracitrovorus in milk plus citric acid	63.2	53.32	53.27	53.295	Acetic plus small amount of propionic
S. paracitrovorus in milk plus citric acid	61.2	53.53	53.48	53.505	Acetic plus trace of propionic
S. citrovorus in milk plus citric acid	50.0	53.06	53.11	53.085	Acetic plus small amount of propionic
S. citrovorus in milk plus citric acid	47.0	53.68	53.58	53.63	Acetic plus trace of propionic
S. citrovorus in milk plus citric acid	43.0	53.63	53.55	53.59	Acetic plus trace of propionic
S. citrovorus in milk plus citric acid	46.0	53.53	53.48	53.505	Acetic plus trace of propionic

*See table IX.

TABLE XI—BARIUM AND DUCLAUX VALUES OBTAINED ON DISTILLATES FROM OLD CREAM SOURED NATURALLY

Cream No.	Incubation	Acidity	Acidity of 1 *l.* dist. from 900 cc. cream (steam distillation)	% Ba in barium salt			Results of Duclaux
				Det. A	Det. B	Av.	
1	R. T. 3 days in July		56.00 cc.	49.14	48.97	49.055	Acetic plus large amount propionic plus tr. butyric
2	R. T. 3 days in July		87.60 "	51.83	51.81	51.82	Acetic plus small amount propionic plus tr. butyric
3	R. T. 4 days in July		48.50 "	48.79	48.73	48.76	Acetic plus large amount propionic plus tr. butyric
4	R. T. 5 days in July		40.75 "	49.44	49.10	49.27	Acetic plus large amount propionic plus tr. butyric
5	R. T. 4 days in July	.90	53.50 "	51.75	51.89	51.82	Acetic plus small amount propionic plus tr. butyric
6	R. T. 10 d. in Nov. & Dec.		81.00 "	48.91		48.91	Acetic plus large amount propionic plus tr. butyric
7	R. T. 6 days in Dec.		50.75 "	51.82	51.66	51.74	Acetic plus small amount propionic plus tr. butyric
8	R. T. 6 days in Dec.		81.50 "	49.66	49.69	49.675	Acetic plus large amount propionic plus tr. butyric
9	R. T. 7 days in Dec.	.80	46.00 "	48.94	49.00	48.97	Acetic plus large amount propionic plus tr. butyric
10	R. T. 6 days in Dec.	.87	49.50 "	51.83	51.88	51.855	Acetic plus small amount propionic

DISCUSSION OF RESULTS

The results presented show that in a highly ripened starter the volatile acid is largely acetic, with undoubtedly a small amount of propionic acid. These volatile acids must be assumed to be of considerable importance from the standpoint of determining the odor and flavor of a starter, altho by no means the only factor. It is evident that the kind of volatile acid present is not the same thruout the ripening period of a starter, the acetic acid being less prominent early in the ripening and more prominent later, and the propionic acid accordingly more prominent early and less prominent later. The acetic acid present in a starter seems to result mainly from the action of the associated organisms, while the propionic acid comes largely from the action of the *S. lactis* group. The time of the appearance of these different types of volatile acids suggests that during the early part of the ripening of a starter the *S. lactis* organisms are the main ones developing and that later on the associated organisms become active. This same idea is suggested by the results previously reported by the Iowa station[3] showing that the percentage of the total acidity made up of volatile acid is greater late in the ripening period than early.

The data secured on the distillates from old sour cream show that the kinds of volatile acids produced during prolonged natural souring are quite different than those produced in the ripening of starters, being higher in the series of fatty acids. These acids are undoubtedly partly responsible for the very undesirable flavors and odors in old cream and in the butter made from it. The results obtained in connection with the kinds of volatile acids produced in cream held for a long period suggest that certain organisms growing in such material may be of quite different types than those ordinarily considered to be the most prominent in causing changes in milk or cream.

3. Hammer, B. W., and Cordes, W. A. The Relation Between the Volatile and Total Acidity in Starters and in Cultures of S. lacticus. Ia. Agr. Expt. Sta. Res. Bul. 66, July, 1921.

CONCLUSIONS

1. Starters ripened for considerable periods contained volatile acids made up largely of acetic with small amounts of propionic.

2. At low total (and accordingly volatile) acidities propionic acid made up a greater percentage of the volatile acidity than it did at higher total acidities.

3. The change from a volatile acidity in which propionic acid was present in comparatively large amounts to one where it made up only a small percentage of the total volatile acid did not occur at any definite total acidity of a starter.

4. *Streptococcus lactis* produced a volatile acidity made up of acetic with considerable amounts of propionic acid.

5. The associated organisms—*S. citrovorus* and *S. paracitrovorus*—produced a volatile acidity that was very largely acetic.

6. The volatile acids secured from old cream were quite different than those secured from starters in that acid higher than acetic was present in large amounts. In some instances butyric acid was apparently included.